REMARKS / ARGUMENTS

Claims 88-95 are canceled, claims 38 and 81 are amended and claim 96 is newly added.

Support for Amendments

Support for amendments to the claims may be found throughout the application including the specification as filed. Exemplary support for each amendment is provided for the convenience of the examiner.

Claim 38 is amended to recite that the fucosyl transferase and xylosyl trasferase are an endogenous alpha 1,3-fucosyl transferase and an endogenous beta 1,2-xylosyl transferase. Support may be found throughout example 2 beginning at page 31, which demonstrates cloning of the alpha 1,3-fucosyltransferase from *P. patens* and analysis of the knockout plants. Further support may be found throughout example 3 beginning at page 38, which demonstrates cloning of the beta 1,2-xylosyltransferase from *P. patens* and analysis of the knockout plants.

Claim 38 is also amended to substitute "wherein" for "whereby" to further clarify a relationship between the structure of the disrupted sequences with the inability to form N-linked glycans with 1,3-linked fucosyl and 1,2-linked xylosyl residues. A typographical error is also corrected.

Claim 81 is amended to ensure proper antecedent basis from claim 38

Claim 96 is newly added, depends from claim 38 and adds that the endogenous alpha 1,3-fucosyl transferase encoding nucleotide sequence is represented by a partial cDNA that has been deposited with GenBank under accession number AJ429145, and wherein the endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence is represented by a cDNA corresponding to the coding region that has been deposited with GenBank under accession number AJ429144. Support may be found at page 32, lines 16-17, which provide, "[the] complete 1711bp of the cDNA for the alpha 1,3-FT (GenBank: partial cDNA: AJ429145)." Further support may be found at page 38, lines 33-34, which provide, "[the] complete 2300 bp of the cDNA for beta 1,2-XT (GenBank: 1788 bp corresponding to the coding region: AJ 429144)."

Introduction of the Invention

The present invention is directed towards a transformed bryophyte cell from *Physcomitrella patens* that is incapable of forming N-linked glycans with 1,3-linked fucosyl and 1,2-linked xylosyl residues. The inventors have surprisingly found that glycosylation in *Physcomitrella patens* resulting in 1,3-linked fucosyl residues and 1,2-linked xylosyl residues are each encoded by a single gene, namely the single genes encoding alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase. The inventors also surprisingly found that a *Physcomitrella patens* cell having a double knockout of alpha 1,3-fucosyl transferase and beta 1,2 xylosyl transferase remains viable. The results were unexpected since N-linked glycoslylation is conserved and disruption of GNT1, another gene involved in N-linked glycosylation, was not able to prevent N-linked glycosylation with N-acetylglucsaminyl residues. Accordingly, the inventors have discovered that a *Physcomitrella patens* cell incapable of forming N-linked glycans with 1, 3-linked fucosyl and 1, 2-linked xylosyl residues may be produced using techniques commonly performed by the skilled artisan.

Specifically, in the claims a transformed bryophyte cell from *Physcomitrella patens* is provided which includes a disrupted endogenous alpha 1,3-fucosyl transferase encoding nucleotide sequence and a disrupted endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence, whereby the bryophyte cell is incapable of forming N-linked gylcans with 1,3-linked fucosyl and 1,2-linked xylosyl residues. Thus claims are directed towards a double knockout of the endogenous alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase in *Physcomitrella patens*. Since each is encoded by a single gene, their disruption resulted in the inability to form N-linked glycans with 1,3-linked fucosyl and 1,2-linked xylosyl residues.

Claim Objections

The examiner indicates claims 88 and 89-95 as provided in the response to Office Action dated 04/22/2008 are a substantial duplicate of pending claims 38 and 39, 40, 42, 48, 50 and 78 and thus would be objected to if the latter are deemed allowable.

Claims 88 and 89-95 are canceled in the present amendment to expedite allowance of claims 38-40, 42, 48, 50 and 78.

Response to Claim Rejections 35 U.S.C. § 112

I.

Enablement Rejections

The examiner rejects claims 38-40, 42, 48, 50, 78 and 81-95 under 35 U.S.C. § 112, first paragraph, as not being enabled. Specifically, the examiner argues the specification does not reasonably provide enablement for a) a transformed bryophyte from *Physcomitrella patens* comprising a disruption of any endogenous fucosyl transferase and xylosyl transferase of undefined structure, and c) said bryophyte cell further comprising human beta 1,4 galactosyltransferase capable of producing human glycosylation pattern.

In particular, the specification according to the Examiner does enable for a specific bryophyte *Physcomitrella patens* wherein in said *P. patens*:

> (i) the endogenous gene encoding for alpha 1,3-fucosyltransferase (FucT, 1711bp, GenBank: partial cDNA: AJ429145, page 32 of specification) is disrupted through targeted insertion by primer sequences SEQ ID NO: 48, 49, 50 and 51, or the endogenous gene encoding for beta 1,2-xylosyltransferase (XylT, 1788bp. GenBank: corresponding to the coding region: AJ429144, page 38 of specification) is disrupted through targeted insertion by primer sequences SEQ ID NO: 67, 68, 69 and 70 (page 39 of specification), or for a double knockout comprising said FucT and XylT disrupted through targeted insertion (double knockout, disrupting the coding regions of said genes, pages 44-45 of specification),
>
> (ii) further in said single or double knockout *P. patens* the gene encoding the human beta 1,4-galactosyltransferase catalyzing the following glycosylation pattern; UDP-galactose + N-acetyl-D-glucosaminylglycopeptide <> UDP + beta-D-galytosyl-1,4-N-acetylbeta-D-

glucosaminylglycopeptide (GalT, GenBank X55415) has been integrated by homologous recombination

(iii) and said *P. patens* comprising said gene knockout (FucT and XylT or double knockout = (i)) and expressing the human galT (=(ii))

(iv) is transformed with an expression construct encoding the secretable/soluble form of human vascular endothelial growth factor (VEGF).

Applicants respectfully request the rejections be withdrawn and the claims allowed in view of the arguments set forth below.

A. The standard for enablement under 35 U.S.C. 112, first paragraph

The enablement requirement is met when the specification teaches one of ordinary skill in the art how to make and use the invention. The general policy of the enablement requirement is to ensure the claimed invention is communicated in such a way that the public may understand and perhaps build on it. However, a detailed report regarding how to make and use the invention is unnecessary if a person of ordinary skill in the art could understand the invention without such an explanation. For instance the CCPA has stated that "not every last detail is to be described, else patent specifications would turn into production specifications, which they were never intended to be." In re Gay, 309 F.2d 769 (C.C.P.A. 1962). This is consistent with the discussion provided in Ajinomoto Co., Inc. v. Archer-Daniels-Midland Co., 228 F.3d 1338 (Fed. Cir.), *cert denied*, 532 U.S. 1019 (2001), which stated,

> "Requiring inclusion in the patent of known scientific/technological information would add an imprecise and open-ended criterion to the content of patent specifications, could greatly enlarge the content of patent specifications and unnecessarily increase the cost of preparing and prosecuting patent applications, and could tend to obfuscate rather than highlight the contribution to which the patent is directed. A patent is not a scientific treatise, but a document that presumes a readership skilled in the field of the invention."

In addition, there is no requirement that, for a patent claim to be enabled, it must enable all embodiments of the invention. On the contrary, the CCPA and Federal Circuit have both recognized that a claim need not enable all embodiments of the invention. Instead, to be enabling, the specification must teach those skilled in the art how to make and use the invention without undue experimentation. Genentech, Inc. v. Novo Nordisk, A/S, 108 F.3d 1361, 1365, 42 USPTO2d 1001, 1004 (Fed. Cir.), *cert. denied*, 522 U.S. 963 (1997). Factors considered whether undue experimentation is required include 1) the quantity of experimentation necessary, 2) the amount of direction or guidance provided, 3) the presence or absence of working examples, 4) the nature of the invention, 5) the state of the prior art, 6) the relative level of skill of those in the art, 7) the predictability of the art, and 8) the breadth of the claims. In re Wands, 858 F.2d 731 (Fed. Cir 1988).

B. With respect to all claims, the examiner argues that the specification does not enable a transformed bryophyte from *Physcomitrella patens* comprising a disruption of any endogenous fucosyl transferase and xylosyl transferase of undefined structure; however, central to the study was the identification that endogenous alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase are each encoded by a single gene in *Physcomitrella patens* and thus only disruption of both endogenous alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase encoding nucleotide sequences is required

At page 6 of the Office Action the examiner argues the guidance is insufficient, as the breadth and scope of the bryophyte cell *Physcomitrella patens* comprising modifications to a genus of structurally undefined genes (diverse structures for genes associated with fucosyl transferase and xylosyl transferase functions) that are disrupted. Further, on page 6, the examiner cites the specification (page 6, ll. 10-15) as admitting that N-glycosylation is very complex and well regulated as N-glycosylation depends not only on developmental stages for plants but also dependent upon culture conditions. Thus, the examiner concludes that the skilled artisan requires information regarding the gene structure, organization and its regulatory elements of all endogenous genes encoding the polypeptides involved in N-glycan synthesis and

amenable for disruption without affecting the viability of the modified/transformed plants and the method for transformation including specific structures such as specific primer sequences for disrupting FucT or XylT.

While the examiner argues the specification does not establish diverse structures of genes associated with fucosyl transferase (FucT) and xylosyl transferase (XylT) functions in *Physcomitrella patens*, and that *Physcomitrella patens* can potentially comprise multiple distinct genes comprising distinct structures and encoding polypeptides with distinct structures having FucT and XylT activities, applicants note that the specification reports that endogenous alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase are each encoded by a single gene. Since there is only one gene for each of alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase in *P. patens*, disruption of each single copy gene resulted in the inability to detect 1,3-linked fucosyl and 1,2-linked xylosyl residues. In other words, the specification demonstrates there are not diverse structures of genes having alpha 1,3-fucoysl transferase and beta 1,2-xylosyl transferase activities in *P. patens*. If there were the experiments would have shown the presence of 1,3-linked fucosyl and 1,2-linked xylosyl residues in spite of the double knockout. In fact, this was surprisingly found since this was not the case in GNT1, as will be discussed.

In fact, the inventors first expected various isoforms or genes to be present, which would provide a complex system as suggested by the examiner. This was in part believed because a complex system was identified in the case of N-acetylglucosaminyltransferase I (GNT1). GNT1 is also involved in N-glycosylation. When disrupting a GNT1 nucleotide sequence, loss of transcript was confirmed; however, only minor differences were found in the glycosylation pattern. For example, referring to page 5, lines 1 to 20, which provides,

> "Moreover, *Physcomitrella patens* shows highly efficient homologous recombination in its nuclear DNA, a unique feature for plants, which enables directed gene disruption ...However, the use of this mechanism for altering glycosylation patterns has proven to be problematic, as shown herein in the examples. Disruption of N-acetylglucosaminyltransferase I (GNT1) in *Physcomitrella patens* resulted in the loss of the specific transcript but only in minor differences of the N-glycosylation pattern...Thus, the knockout in *Physcomitrella patens* did not result in the expected modification of the N-glycosylation pattern."

In example 1 on page 31, the results from the GNT1 experiments were summarized,

> "Three GNT1 knockout plants were analysed. The N-glycans of GNTI knockout plants exhibited the same structures compared to WT (tab. 1) confirming that the knockout was only successful on the molecular but not on the biochemical level. Therefore, it is assumed that another GNTI exists in *Physcomitrella patens*." (emphasis added)

Thus, while the GNT1 specific transcript was lost, biochemically, N-linked glycosylation was not significantly affected. This leads one to believe that the mechanism for such glycosylation is more complex than a single gene. Given the difficulties in the experiments with GNT1, it was initially believed a knockout of alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase would still result in alpha 1,3-fucosyl transferase activity and beta 1,2-xylosyl trasferase activity. In other words, it was believed that there were additional copies of the gene or isoforms and 1,3-linked fucosyl residues and 1,2-linked xylosyl residues were expected to be present in spite of the disruption of nucleotide sequences encoding alpha 1,3-fucsoyl transferase and beta 1,2-xylosyl transferase. The inventors were surprised to find that knockouts were successful. That is, 1,3-linked fucosyl residues and 1,2-linked xylosyl residues were not detected. This is summarized at page 5, lines 21-31, which provides,

> "Although the knockout strategy was not successful for GNT1, the present inventors attempted to knock out the beta 1,2-xylosyltransferase (XylT) and alpha 1,3-fucosyltransferase (FucT) in *Physcomitrella patens*. Specific transcripts could not be detected in the resulting plants. Surprisingly, the N-linked glycans isolated from the transgenic plants were found to be modified in the desired manner. No 1,3 linked fucosyl residues could be detected on N-linked glycans of FucT knockout plants and no 1,2 linked xylosyl residues could be detected on N-linked glycans of XylT knockout plants.

Further it was surprising to find that the knockout of alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase did not adversely affect growth of the double knockout plants since plant specific glysosylation is highly conserved. This is discussed at page 5, line 31 through page 6, line 5,

> "The isolated transgenic lines showed normal growth which is surprising considering that plant specific glycosylation is highly conserved and therefore would be expected to be significant for function. Double knockouts should

> therefore have been expected to have had a detrimental effect on the growth of the moss. In addition, compensating were expected but surprisingly were not apparent. Moreover, the double knockout of FucT and XylT resulted in modified N-linked glycans without detectable 1,3 linked fucosyl and 1,2 linked xylosyl residues."

Thus, while the examiner presumes there must be diverse structures with respect to fucosyl transferase and xylosyl transferase activities, it was found that this is not the case. Endogenous alpha 1,3-fucoysyl transferase and beta 1,2-xylosyl transferase are each encoded by a single endogenous gene. However, it does appear there are diverse structures of genes associated with GNT1, which is also involved in N-glycosylation.

For completeness, example 2 provides further evidence that genes encoding endogenous alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase are provided as a single copy. In example 2, degenerate primers were used to provide cDNA from fucoysl transferases in *P. patens*. The degenerate primers were created on the basis of protein alignment of known alpha 1, 3-fucsoyl transferases from *Vignia radiate* and *Arabidopsis thaliana*. This approach was used to identify all FucT sequences in *P. patens*. PCR revealed a 510 bp product which was cloned and sequenced from both ends. Subsequent 5' and 3' RACE experiments resulted in "the complete 1711 bp of the cDNA for the alpha 1, 3 – fucosyl transferase (GenBank partial cDNA AJ429145)" (page 37). The corresponding 3083 bp of the genomic sequence was obtained by cloning the alpha 1,3 FT gene in three piece by PCR from WT *P. patens* genomic DNA with specific primers...which were sequences by primer walking. (page 32 lines 19-27).

Once the nucleotide sequence encoding endogenous alpha 1,3-fucosyl transferase was identified, a knockout construct was created using techniques regularly practiced by those skilled in the art in view of a known gene sequence. In this instance the knockout construct replaced a 195 bp fragment of the fourth exon and part of the fifth intron with a nptII cassette. As discussed at page 33, transgenic plants were pre-screened using four pairs of primers to detect (i) disruption of the endogenous alpha 1,3-FucT gene, (ii) the presence of the nptII cassette, (iii) to control the integration of the transgene at the 5' end, and (iv) to control the integration of the transgene at the 3' end. Lines 29-35 state,

> "Plants that gave the expected fragments by all four PCR reactions were considered as putative knockouts and selected for further analysis.

Finally 9 plants were chosen for further molecular and biochemical analysis."

RT-PCR was performed using primers in the central region of cDNA for alpha 1, 3-fucosylt transferase. Referring to lines 7-12,

> "Using these primers a 475 bp transcript was detected only in the WT whereas all transgenic plants did not give any PCR products. The absence of the detectable transcript with the primers located on both sides of integrated nptII cassette confirms that all plants analysed are knockouts."

Once the knockout of the single copy had been confirmed, the question remained whether a knockout of the identified gene would result in N-linked 1,3-fucosyl residues. For instance, while the GNT1 transcript was successfully knocked out, proteins remained glycosylated. Thus, the question remained whether there were diverse structures able to provide 1,3-linked fucosyl residues in spite of the absence of FucT transcript.

MALDI-TOF mass spectrometry was performed to assess whether 1,3-linked fucosyl residues remained in the transformed *P. patens*. On page 34, the results are summarized,

> "Three alpha 1,3 FT knockout plants were analysed. No alpha 1,3 fucose residues linked to the Asn-bound GlcNAc could be detected on the N-glycans of alpha 1,3 FT knockout plants (tab. 1) confirming that the knockout of alpha 1,3 fucosyltransferase in *Physcomitrella patens* was completely successful."

The same result was found when testing for the knockout of beta 1,2-xylosyl transferase activity after providing a knockout of the beta 1,2-xylosyl transferase single copy gene, at page 41,

> "Three beta 1, 2XT knockout plants were analysed. No beta 1,2 xylosyl residues linked to the beta-mannosyl residue could be detected on the N-glycans of beta 1,2 XT knockout plants (tab. 1) confirming that the knockout of beta 1,2 xylosyltransferase in Physcomitrella patens was completely successful."

Since only one transcript could be found in wild type plants for each of alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase and no such transcript could be found in the knockout plants, it was clear that there existed only one copy of each gene in the genome of *P. patens*.

As further evidence that the gene encoding each of alpha 1,3-fucosyl transferase and beta

1,2-xylosyl transferase exist in single copies in *Physcomitrella patens*, Applicants provide herewith a copy of Kaprivova et al., (Plant Biotechnology 2004), which at page 520, col. 2, last paragraph through page 521 col. 1 states,

> "Indeed MALDI spectra clearly proved the disappearance of these residues from the N-glycan structures of the corresponding knockouts. Obviously, Fuc-T and Xyl-T are encoded by single copy genes and no other moss enzyme can substitute them. Despite the conserved character of these two sugar resides in the whole plant kingdom, the 1,3-fucose and 1,2-xylose of plant N-glycans are dispensable under our growth conditions, as plants lacking Fuc-T or Xyl-T activity were not impaired in growth and morphology." (emphasis added)

Thus, while the examiner argues the breadth and scope of the bryophyte cell *Physcomitrella patens* comprises modifications to a genus of structurally undefined genes (diverse structures for genes associated with fucosyl transferase and xylosyl transferase functions) that are disrupted, the application clearly shows there exits one copy each of the endogenous alpha 1,3-fucosyl tranferase encoding gene and the beta 1,2-xylosyl transferase encoding gene in *P. patens*. Further, the application shows that effective disruption of the endogenous alpha 1,3-fucosyl transferase encoding nucleotide sequence and the endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence in *P. patens* will result in the inability to detect 1,3-linked fucosyl and 1,2-linked xylosyl residues.

Thus, knowing that there exists only one copy of an endogenous alpha 1,3-fucosyl transferase encoding gene and one copy of an endogenous beta 1,2-xylosyl transferase encoding gene, knowing nucleotide sequence information of each, knowing the function of alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase, and knowing 1,3-fucosyl transferase and 1,2-xylosyl transferase are not required for cell viability, one skilled in the art can use well known techniques to disrupt the alpha 1,3-fucosyl transferase encoding nucleotide sequence and beta 1,2-xylosyl transferase encoding nucleotide sequence to produce a transformed *Physcomitrella patens* cell that is incapable of producing N-linked glycans with 1,3-linked fucosyl residues and 1, 2-linked xylosyl residues. Further, given the instructions how to produce such a *Physcomitrella patens* cell and how to test for success, its production would not require undue experimentation.

C. With respect to all claims and the Wands factors, undue experimentation would not be required to enable the skilled artisan to practice the invention

The examiner cites In re Wands and concludes the specification does not commensurate with the scope of the claims because it would require undue experimentation. The determination of whether an invention requires undue experimentation is not based on a single factor, but is rather a conclusion reached by weighing several factors. A set of eight (8) factors were provide in In re Wands and are listed by the examiner at page 4 of the Office Action. However, it should be noted that the factors are "illustrative, non mandatory. What is relevant depends on the facts." Amgen, inc. v. Chugai Pharm. Co., 927 F.2d 1200, 1213 (Fed. Cir 1991) *cert denied*, 502 US 856 (1981).

As will be demonstrated, consideration of the Wands factors supports the position that the specification clearly enables a transformed *Physcomitrella patens* cell including a disrupted endogenous alpha 1,3-fucosyl transferase encoding nucleotide sequence and a disrupted endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence, whereby the bryophyte cell is incapable of forming N-linked glycans with 1,2-linked fucosyl and 1,2-linked xylosyl residues. Applicants incorporate by reference the description set forth above.

With respect to the first Wands factor, the quantity of experimentation necessary, the application demonstrates in the examples the disruption of an endogenous alpha 1,3-fucoysl transferase encoding nucleotide sequence and an endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence that resulted in the inability of *Physcomitrella patens* to form N-glycans with alpha 1,3-linked fucosyl and beta 1,2-linked xylosyl residues. Thus, examples are provided which demonstrate the generation of a double knockout of alpha 1,3-fucosyl transferase and beta 1,2 xylosyl transferase. Given the instructions of how to provide the disruptions to form a double knockout of 1,3-fucosyl transferase and beta 1,2-xylosyl transferase and as shown above, given examples of how to confirm the lack of N-linked glycans with 1,3-linked fucosyl and 1,2-linked xylosyl residues, and given the knowledge that alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase are not required for cell viability, clearly one skilled in the art would not need undue experimentation to obtain a transformed *P. patens* cell having a disrupted

endogenous alpha 1,3-fucosyl transferase encoding nucleotide sequence and a disrupted endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence, whereby the bryophyte is incapable of forming N-linked glycans with 1,3-linked fucosyl and 1, 2-linked xylosyl residues. Again, the specification provides instructions, including exemplary sequences, which demonstrate how to make the invention and how to test for success.

With respect to the second Wands factor, the amount of direction or guidance presented, the specification includes examples that demonstrate the disruption of an endogenous alpha 1,3-fucoysl transferase encoding nucleotide sequence and an endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence resulting in the inability of *Physcomitrella patens* to form N-glycans with alpha 1,3-linked fucosyl and beta 1,2-linked xylosyl residues. It is also reported in the specification that both enzymes (alpha 1,3-FucT and beta 1,2-XylT) that modify the N-glycan structure by introducing alpha 1,3-linked fucosyl and beta 1,2-linked xylosyl residues are not required for cell viability. Given the instructions of how to provide the disruptions to construct a double knockout of alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase and given examples of how to confirm the lack of N-linked glycans with 1,3-linked fucosyl and 1,2-linked xylosyl residues, and given the knowledge that both enzymes (alpha 1,3-FucT and beta 1,2-XylT) are not required for cell viability, clearly extensive direction and guidance is presented for one skilled in the art to obtain a transformed *P. patens* cell having a disrupted endogenous alpha-1,3-fucosyl transferase encoding nucleotide sequence and a disrupted endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence, whereby the bryophyte (*P. patens*) is incapable of forming N-linked glycans with 1,3-linked fucosysl and 1, 2-linked xylosyl residues. Still further, the application on pages 7 and 8 indicate the methods may be performed using targeted deletions as well as substitutions discussed herein.

With respect to the third Wands factor, the presence or absence of working examples, the specification provides an examples section demonstrating the disruption of an endogenous alpha 1,3-fucoysl transferase encoding nucleotide sequence and an endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence that resulted in the inability of *Physcomitrella patens* to form N-glycans with alpha 1,3-linked fucosyl and beta 1,2-linked xylosyl residues. Thus, the examples demonstrate to one skilled in the art how to provide disruptions in the endogenous alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase resulting in a double knockout and

how to confirm the intended result.

With respect to the fourth Wands factor, the nature of the invention, the nature of the invention is a transformed *Physcomitrella patens* which includes a disrupted endogenous alpha 1,3-fucosyl transferase encoding nucleotide sequence and a disrupted beta 1,2-xylosyl transferase encoding nucleotide sequence (or alpha 1,3-FucT and beta 1,2-XylT double knockout) that is incapable of forming N-linked glycans with 1,3-linked fucosyl and 1,2-linked xylosyl residues. As demonstrated above, the endogenous alpha 1,3-fucosyl transferase and beta 1,2 xylosyl transferase are each encoded by a single copy of a gene. Thus, in this instance the double knockout only requires disruption of each single copy. Thus, while the invention is related to genetically modified plant cells, the genetic modification only requires disruption at each of two single copy genes. Such procedures are often performed in research laboratories.

With respect to the fifth Wands factor, the state of the prior art, the state of the prior art is quite high. Disrupting a single copy of an endogenous nucleotide sequence in a plant cell is commonplace. Further, kits and apparatuses to obtain and sequence DNA are commonly available as are apparatuses that may be used to test for the presence of 1,3-linked fucosyl residues and 1,2-linked xylosyl residues. Laboratories regularly perform PCR, MALDI-TOF mass spec, and various cloning techniques, which are often further facilitated by commercially available kits.

With respect to the sixth Wands factor, the relative skill of those in the art, the skill in the present art is quite high. Numerous laboratories construct genetic knockouts in plants. Techniques such as PCR, RT-PCR, MALDI-TOF mass spectrometry, sequencing and cloning techniques are common skills to those in the present art. Further, commercial kits and programs are common to provide additional support.

In summary:

A) given demonstrations through examples of how to generate a transformed bryophyte cell from *Physcomitrella patens* that comprises i) a disrupted endogenous alpha 1,3-fucosyl transferase encoding nucleotide sequence and ii) a disrupted endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence, whereby the bryophyte cell is incapable of forming N-linked glycans with 1,3-linked fucosysl and 1, 2-linked xylosyl residues;

B) given examples how to confirm the presence of a transformed bryophyte cell from *Physcomitrella patens* that comprises i) a disrupted endogenous alpha 1,3-linked fucosyl transferase nucleotide sequence and ii) a disrupted endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence, whereby the bryophyte cell is incapable of forming N-linked glycans with 1,3-linked fucosysl and 1, 2-linked xylosyl residues;

C) given the knowledge that alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase are not required for cell viability in *Physcomitrella patens*; and

D) given the high level of skill of the skilled artisan and advanced state of the art;

one skilled in the art would, without undue experimentation, be able to provide a transformed bryophyte cell from *Physcomitrella patens* that comprises i) a disrupted endogenous alpha 1,3-fucosyl transferase encoding nucleotide sequence and ii) a disrupted endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence, whereby the bryophyte cell is incapable of forming N-linked glycans with 1,3-linked fucosysl and 1, 2-linked xylosyl residues. Should there be any confusion the skilled artisan can follow the examples. Thus, in view of the Wands factors as set forth by the examiner the claims are enabled.

D. With respect to all claims, given a single gene sequence that produces a protein with a known activity, those skilled in the present art would be able to construct and confirm the knockout of the gene and its activity.

The examiner argues that the specification must provide a rational and predictable scheme for modifying any amino acid residue or the respective codon in the polynucleotide with an expectation of obtaining the desired function and guidance as to which of the essentially

infinite possible choices is likely to be successful. (Office Action page 7).

First, applicants refer to the above discussion that alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase are each encoded by a single gene. Construction of a genetic knockout of a single copy gene in plants is not new. In other words, referring to the Wands factors, both the state of the art and the relative skill of those in the art are quite high. Thus, given a known single copy gene, its function, a method to test for activity, and the knowledge that both alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase are not required for cell viability in *Physcomitrella patens*, it is clear one skilled in the art can practice the invention provided in the claims.

Further, while the examiner argues guidance is required as to the "infinite possible choices", which is likely to be successful, success is explicitly provided in the examples section. Clearly, the examples demonstrate the successful production of a *Physcomitrella patens* cell having a disrupted endogenous alpha 1,3-fucosyl transferase encoding nucleotide sequence, and a disrupted endogenous beta 1,2-xylosyl transferase encoding nucleotide sequence, whereby the bryophyte is incapable of N-linked glycosylation with 1,3-fucosyl and 1,2-linked xylosyl residues. Thus, one skilled in the art can refer to the examples for a specific method.

E. With respect to all claims, although the examiner requires the specification provide regions of the protein/polypeptide structure which may be modified without affecting the viability of a bryophyte following disrupting any endogenous gene encoding fucosyl transferase and xylosyl transferase in *Physcomitrella patens*, the specification shows that alpha 1,3-fucsosyl transferase and beta 1,2-xylosyl transferase are not necessary for cell viability of *Physcomitrella patens*

The examiner raises a concern regarding which regions of the protein or polypeptide structure may be modified without affecting the viability of a bryophyte. However, the present invention demonstrates that alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase in *P. patens* are not required for viability. Specifically, the double knockout was shown to be viable. Further, in the examples, *P. patens* is viable without alpha 1,3 fucosyl transferase and beta 1,2

xylosyl transferase.

F. With respect to broad galactosyltransferase rejections, human beta 1,4 galactosyltransferase is claimed and enabled

The examiner argues that any given galactosyltransferase activity is specific and limited to certain specific substrates and its spectrum of activity is also limited and specific. Although cited in the rejection of all claims, this is presumed to be in response to claims 42, 83 and 89 which claim a human beta 1, 4 galactosyltransferase that is expressed in the bryophyte cell (*P. patens*). Claims 42 and 83 (89 canceled herein) claim a specific galactosyltransferase, namely human beta 1,4 galactosyltransferase. Further, the examiner acknowledges that human beta 1,4 galactosyltransferase is enabled in the rejection (page 3).

II.
Written Description Rejections

The examiner rejected claims 38-40, 42, 48, 50, 78 and 81-95 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The examiner argues there are no structural limitations or structure-function correlation recited in the claims with respect to a transformed bryophyte from *Physcomitrella patens* comprising disruption of any of endogenous fucosyl transferase and xylosyl transferase of undefined structure. Specifically, the examiner argues application fails to provide any information as to a transformed bryophyte from *Physcomitrella patens* comprising extremely large number of FucT or XylT polynucleotides and encoding polypeptides of undefined structure as broadly encompassed by the claims as the *Physcomitrella patens* can potentially comprise multiple distinct genes comprising distinct structures and encoding polypeptides with distinct structures having FucT or XylT activities.

Applicants respectfully request the rejections be withdrawn and the claims allowed for the reasons set forth below.

A. The standard for written description

The written description requirement places in possession of the public what the applicant considers to be the invention for which a patent is being sought. As stated by the examiner (Quoting Univ. of Calif. V. Eli Lilly), "A written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula, [or] chemical name,' of the claimed subject matter sufficient to distinguish it form other materials." The examiner also refers to MPEP § 2163, in that the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings or by disclosure of relevant identifying characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that Applicant was in possession of the claimed genus.

Further, the examiner cites MPEP § 2163 as stating that a representative number of species means that the species which are adequately described are representative of the entire genus and thus when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

B. **With respect to all claims, the examiner has failed to show there is a substantial variation among the species within alpha 1,3 fucosyl transferase and beta 1,2 xylosyl transferase in *Physcomitrella patens*, and the specification demonstrates that in *Physcomitrella patens* there exists only one copy of each of an endogenous alpha 1,3-fucoysl transferase encoding gene and beta 1,2-xylosyl transferase encoding gene**

The examiner raises a concern that there are multiple distinct genes comprising multiple distinct structures and encoding polypeptides with distinct structures having FucT or XylT activities. Thus in essence, the examiner argues that fucosyl transferase and xylosyl transferase represent a genus of many species. That is, there may be other structures that perform the same

function, whether known or unknown that possess the same function, which is the N-linked glcosylation of 1,3-fucosyl and 1,2-linked xylosyl residues.

As discussed above, the specification demonstrates that in *P. patens* alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase are each encoded by a single gene. Also as discussed above, the specification further shows after disrupting the alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase encoding genes in *P. patens* to form a double knockout, 1,3-linked fucosyl and 1,2-linked xylosyl residues are not detected. Thus, the knockout of alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase results in *P. patens*' inability to form N-linked glycans with 1,3-linked fucosyl and 1,2-linked xylosyl residues. In other words, knockout of each of the single copies of genes encoding alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase results in the inability to form 1,3-linked fucosyl and 1,2-linked xylosyl residues.

Further, in the claims, the endogenous alpha 1,3-fucosyl transferase encoding nucleotide sequence and the endogenous beta 1,2-xylosysl transferase encoding nucleotide sequence are limited to those in *P. patens*, which is shown to only have one copy of each. That is, the claims are not directed to all bryophytes but *P. patens*. Thus, while the examiner presumes there are a number of structures having fucosyl transferase and xylosyl transferase activity, the present application reports that endogenous alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase are each encoded by a single gene in *P. patens*.

The examiner adds that proteins that have similar structures can have different activities and that functionally similar molecules can have different structures. Thus, the examiner presumes that fucosyl transferase and xylosyl transferase activities may have a wide variety of structures. However, applicants demonstrate the inability of *P. patents* to form 1,3-linked fucosyl and 1,2-linked xylosyl residues after disrupting the single copy of each of the nucleotide sequence encoding alpha 1,3-fucosyl transferase and beta 1,2 xylosyl transferase. Thus, it is unclear what alternative molecules would perform N-linked glycosylation with 1,3-linked fucosyl or 1,2-linked xylosyl residues.

For clarity, Applicants have substituted the term "wherein" with "whereby" in the claims to further clarify the relationship between disrupted alpha 1,3-fucosyl transferase and beta 1,2-xylosyl transferase encoding nucleotide sequences with the inability to form 1,3-linked fucosyl and beta 1,2-linked xylosyl residues.

Conclusion

In view of the amendments and arguments set forth above, Applicants respectfully request the withdrawal of each rejection and the request a Notice of Allowance be issued for the instant application. Question regarding the invention may be direct to the attorney of record.

Respectfully submitted,

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